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Apolipoprotein L1 Variant Associated with Increased Susceptibility to Trypanosome Infection

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ABSTRACT African trypanosomes, except *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*, which cause human African trypanosomiasis, are lysed by the human serum protein apolipoprotein L1 (ApoL1). These two subspecies can resist human ApoL1 because they express the serum resistance proteins *T. b. gambiense* glycoprotein (TgsGP) and serum resistance-associated protein (SRA), respectively. Whereas in *T. b. rhodesiense*, SRA is necessary and sufficient to inhibit ApoL1, in *T. b. gambiense*, TgsGP cannot protect against high ApoL1 uptake, so different additional mechanisms contribute to limit this uptake. Here we report a complex interplay between trypanosomes and an ApoL1 variant, revealing important insights into innate human immunity against these parasites. Using whole-genome sequencing, we characterized an atypical *T. b. gambiense* infection in a patient in Ghana. We show that the infecting trypanosome has diverged from the classical *T. b. gambiense* strains and lacks the TgsGP defense mechanism against human serum. By sequencing the ApoL1 gene of the patient and subsequent *in vitro* mutagenesis experiments, we demonstrate that a homozygous missense substitution (N264K) in the membrane-addressing domain of this ApoL1 variant knocks down the trypanolytic activity, allowing the trypanosome to avoid ApoL1-mediated immunity.

IMPORTANCE Most African trypanosomes are lysed by the ApoL1 protein in human serum. Only the subspecies *Trypanosoma b. gambiense* and *T. b. rhodesiense* can resist lysis by ApoL1 because they express specific serum resistance proteins. We here report a complex interplay between trypanosomes and an ApoL1 variant characterized by a homozygous missense substitution (N264K) in the domain that we hypothesize interacts with the endolysosomal membranes of trypanosomes. The N264K substitution knocks down the lytic activity of ApoL1 against *T. b. gambiense* strains lacking the TgsGP defense mechanism and against *T. b. rhodesiense* if N264K is accompanied by additional substitutions in the SRA-interacting domain. Our data suggest that populations with high frequencies of the homozygous N264K ApoL1 variant may be at increased risk of contracting human African trypanosomiasis.

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The human innate immunity is crucial to resist infection with African trypanosomes. Only the subspecies *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* can resist lysis by most human sera and thus cause human African trypanosomiasis (HAT) in western/central and eastern/southern Africa, respectively. *T. b. gambiense* has been historically divided into two groups (1). *T. b. gambiense* group 1 (*T. b. gambiense* 1) is a homog-

enous group of isolates, constitutively resistant to human serum and responsible for the majority of HAT cases. *T. b. gambiense* 2 is a rather ambiguous group of strains which were isolated from patients in the 1970s but do not form a monophyletic group (2) and show varied levels of resistance to human serum (3). *T. b. gambiense* 1 and *T. b. rhodesiense* are pathogenic to humans because they developed resistance mechanisms against apolipo-

protein L1 (ApoL1), the trypanolytic component in human serum (4).

ApoL1 is contained within two serum complexes, trypanolytic factors 1 and 2 (TLF1 and -2) (5, 6). Both TLF1 and TLF2 are able to lyse other trypanosomes, such as *Trypanosoma brucei brucei* and *Trypanosoma evansi*, which cause nagana and surra in animals, respectively, so these trypanosomes are not infectious to humans. TLF1 can be efficiently taken up by trypanosomes through binding to the haptoglobin-hemoglobin (HpHb) receptor (6, 7). However, in normal human serum, the HpHb receptor is saturated with serum HpHb, and ApoL1 enters the trypanosome only via TLF2, independently of the HpHb receptor (6, 7). The exact mechanism behind TLF2 uptake by trypanosomes is still unknown. The trypanolytic activity of ApoL1 results from ionic pore formation in endolysosomal membranes, mediated by the combination of contiguous pore-forming and membrane-addressing domains (8). *T. b. gambiense* 1 and *T. b. rhodesiense* resist ApoL1 through the activities of distinct resistance proteins. *T. b. gambiense* expresses the *T. b. gambiense*-specific glycoprotein (TgsGP), which is necessary but not sufficient for full resistance, and *T. b. rhodesiense* expresses the serum resistance-associated protein (SRA) (9, 10). While TgsGP prevents ApoL1 pore-forming activity by stiffening the target endolysosomal membranes, SRA inhibits ApoL1 by direct interaction with the C-terminal domain of this protein (4, 9, 11). The *TgsGP* gene is physically linked to a truncation of a gene homologous to yeast *AUT1* (*ATG3*), a gene involved in internal vesicular formation (12). *T. b. gambiense* is heterozygous for *AUT1*, with the full and truncated alleles located in independent chromosome II homologues (12). In hypohaptoglobinemic patients, receptor-mediated uptake of TLF1 is efficient due to the absence of the competing HpHb ligand for the HpHb receptor and can overcome the TgsGP-mediated resistance of *T. b. gambiense* 1 (9). Therefore, this parasite is characterized by an L210S substitution in the HpHb receptor, which inhibits TLF1 uptake and allows *T. b. gambiense* 1 infection in regions with a high frequency of hypohaptoglobinemia (6, 9). Two variant ApoL1 alleles (G1 and G2) are frequently observed in West Africans, presumably because these variants resist inhibition by SRA and can thus lyse *T. b. rhodesiense* (13). The G1 ApoL1 genotype is characterized by the coding single nucleotide polymorphisms (SNPs) rs73885319 (S342G) and rs60910145 (I384M) in the SRA-interacting domain, and the G2 ApoL1 genotype is characterized by the allele for indel rs71785313 (N388del and Y389del) in the same domain (13). None of 75 human sera with different combinations of G1 and G2 genotypes were able to lyse *T. b. gambiense* in vitro, as was also observed with recombinant G1 or G2 ApoL1 (13). Both TgsGP and SRA are noncanonical variant surface glycoproteins (VSG), which are routed to the lysosome of the trypanosome, in contrast to functional native VSGs. Native VSGs cover the trypanosome plasma membrane as a dense layer of homodimers and help to evade the host's immune system through continuous variation of surface-exposed antigenic loops (14).

In this work, we report an ApoL1 variant in a patient in Ghana who was infected with an atypical *T. b. gambiense* strain lacking the TgsGP defense mechanism against ApoL1. The ApoL1 variant is characterized by a homozygous N264K missense substitution that knocks down its lytic activity against *T. b. gambiense* devoid of TgsGP.

RESULTS

Patient. In 2013, a 54-year-old man presented at the Ghana Ports and Harbours Authority Hospital in Takoradi City, Ghana, with complaints of gastroenteritis, fever, and nausea. The patient was HIV positive but not on antiretroviral therapy at the time of admission. He was taking co-trimoxazole as prophylaxis for HIV-related opportunistic infections and artemisinin-based combination therapy against malaria. His hemoglobin level was 9.2 g/dl, white blood cell count was 9.3×10^9 /liter (58% neutrophils, 42% lymphocytes), and platelet level was 259×10^9 /liter. Giemsa-stained thin blood smears revealed an infection with trypanosomes, and he was treated with 10 injections of pentamidine at 4 mg/kg of body weight/day intramuscularly for 7 days. No lumbar puncture was performed. He responded well to the trypanocidal treatment, and no trypanosomes were microscopically observed in blood films prepared after treatment. These blood films did show the presence of malaria parasites. Because no HAT cases have been reported in Ghana in the last 10 years, the blood of the patient before treatment was collected on Whatman filter paper for further analysis. Ethical clearance for the study was obtained from the institutional ethics committee of the Institute of Tropical Medicine, Antwerp, Belgium.

Trypanosome genome sequencing and SNP analysis. We extracted the DNA from the dried blood spot, and subsequent whole-genome sequencing generated 10,738,766 paired-end reads of 600 nucleotides (2×300 bp). Most (79.29%) of these reads mapped to the human genome and were discarded. Many (1,509,189) of the remaining reads could be mapped to the *T. b. brucei* TREU927 reference genome, with 10-fold average and 7-fold median coverage. Sixty-two percent of the sample genome (here referred to as the GHANA genome) with a minimum coverage of 5 was used for SNP calling. Quality-scored reads have been submitted to the NCBI Sequence Read Archive (accession number SRP072325). Following mapping of all sequence data, including publically available sequence data from *T. b. gambiense* types 1 and 2, *T. evansi*, and *T. b. brucei*, SAMtools mpileup identified 78,899 SNPs. A RAxML maximum-likelihood tree revealed a single monophyletic clade of *T. b. gambiense* 1 strains [DAL972, 1829(Alijo), and Fontem strain 10] (Fig. 1). The GHANA trypanosome was genetically more related to *T. b. gambiense* 1 than to any other trypanosome in this data set. The GHANA genome differed from the *T. b. gambiense* 1 strains Fontem strain 10, DAL972, and 1829(Alijo) by, respectively, 7,950, 8,233, and 8,050 SNPs and from the *T. b. gambiense* 2 strains TH126 and STIB386 by, respectively, 15,050 and 15,437 SNPs. To exclude the possibility that the GHANA trypanosome originated by recombination of *T. b. gambiense* 1 with another, related trypanosome, a NeighborNet Split network was generated (Fig. S1). Reticulation was limited to the center of the network, and no ambiguous phylogenetic signal related to *T. b. gambiense* 1 and GHANA was observed. In addition, the GHANA sample showed the least number of heterozygous SNPs compared to the other trypanosomes (see Table S2 in the supplemental material). These results suggest that the GHANA trypanosome is not a product of recombination. The gene for the *T. b. gambiense* 1 serum resistance TgsGP protein was completely absent from the GHANA genome. The absence of this gene was further confirmed by PCR analysis with *TgsGP*-internal primers (Fig. S2). Since the *TgsGP* gene is physically linked to a heterozygous truncated *AUT1* allele, we have tested the GHANA DNA by

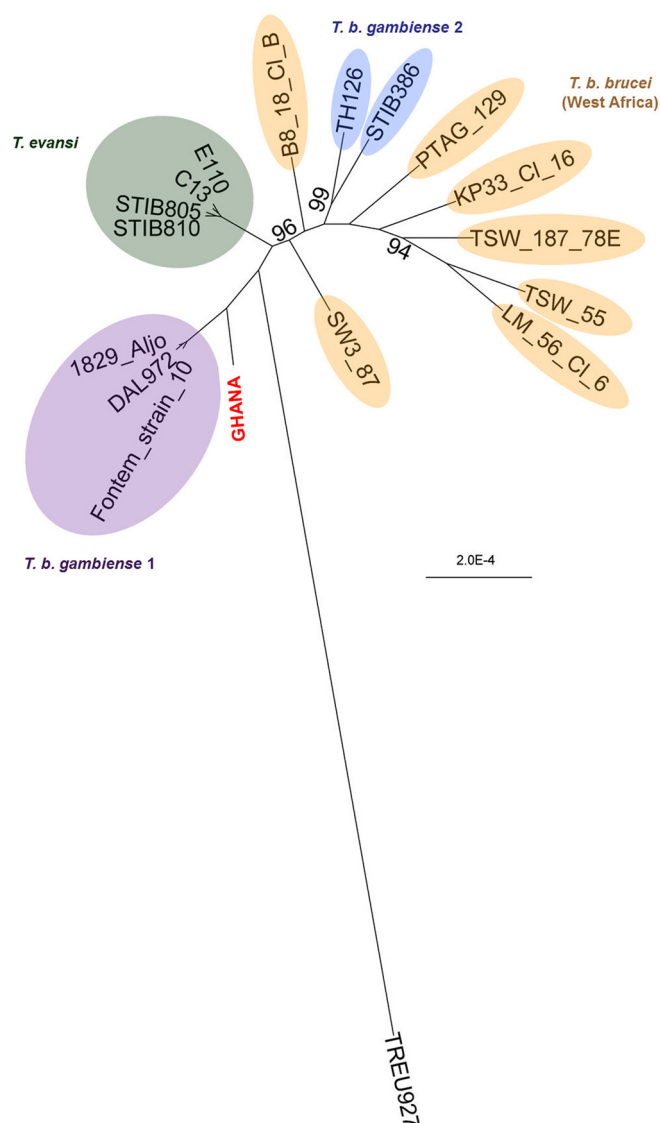


FIG 1 SNP analysis of the trypanosome in the patient and reference strains. Shown is a bootstrapped RAxML maximum-likelihood tree based on 78,899 SNP loci identified in the patient's trypanosome (GHANA) and on publicly available sequence data from three *T. b. gambiense* 1 strains, two *T. b. gambiense* 2 strains, five *T. evansi* strains, seven West African *T. b. brucei* strains, and the East African *T. b. brucei* reference strain TREU927. Bootstrap values were calculated on the basis of 1,000 replicates and are presented in the figure if they were below 100%.

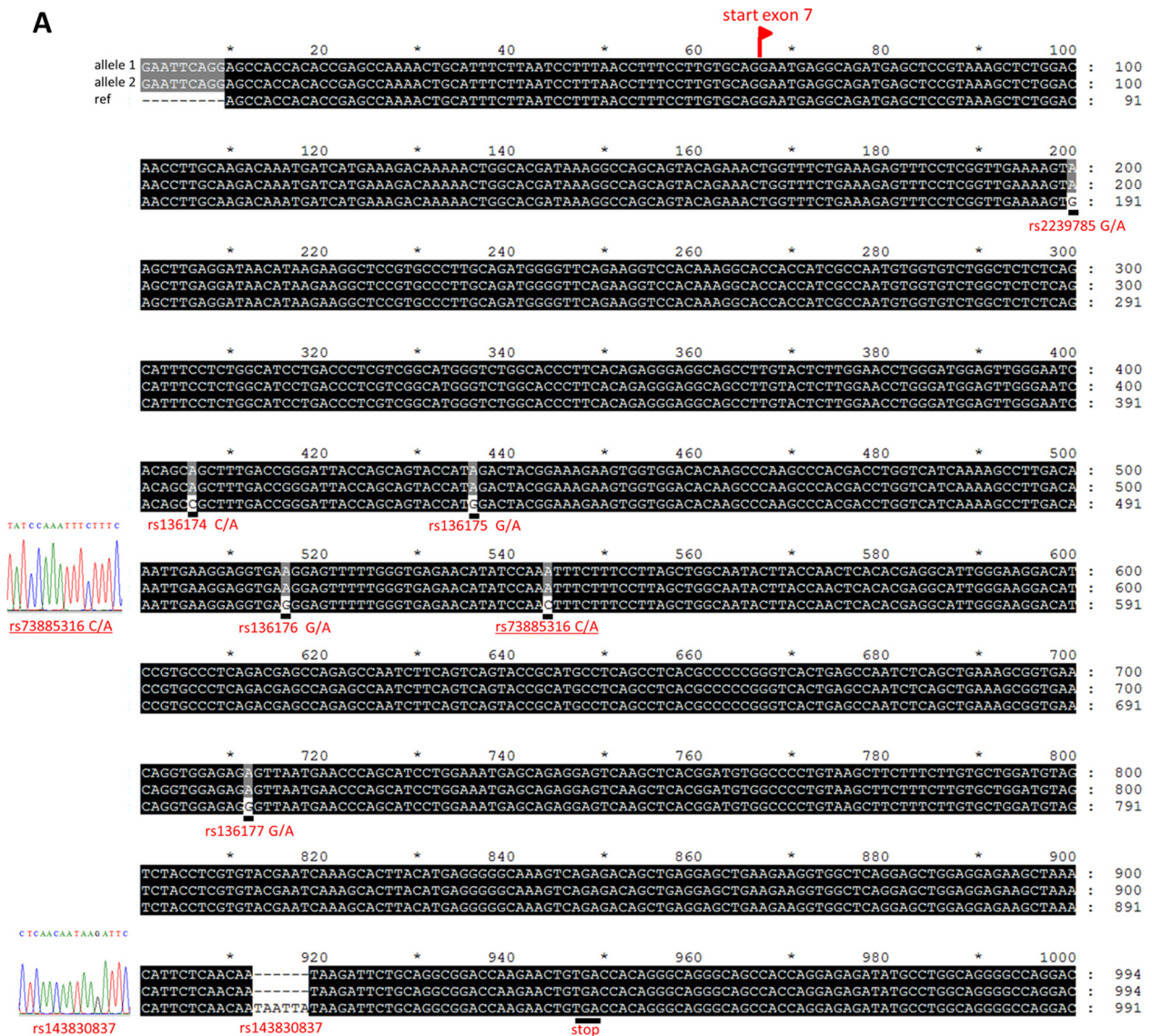
PCR with a primer set that amplifies the full *AUT1* allele and a primer set that amplifies the truncated *AUT1* allele (Fig. S3). GHANA showed a homozygous intact *AUT1* gene, as in *T. b. brucei*. However, the GHANA genome reads that mapped to the reference sequence of the HpHb receptor gene harbored the *T. b. gambiense* 1-specific codon that is responsible for the L210S substitution in the HpHb receptor. Because only one read mapped to this region, we further confirmed this substitution by Sanger sequencing of the complete GHANA HpHb receptor gene. GHANA has the *z1/z1* alleles of the *T. b. gambiense* 1 DAL972 strain (Fig. S4A) described by Symula et al. (15) and has the codon for the L210S substitution in the HpHb receptor (Fig. S4B). This

L210S substitution in the HpHb receptor decreases the binding and internalization of TLF1 in hypohaptoglobinemic-patient serum (9). Given that our patient was anemic and coinfectd with malaria, the reduced uptake of TLF1 via the trypanosome's mutated HpHb receptor partially explains the resistance of the trypanosome to human serum. However, it is known that ApoL1 can still enter the trypanosome via TLF2 and lyse trypanosomes that do not have the TgsGP or SRA resistance proteins against ApoL1 (9).

ApoL1 genotyping and *in vitro* mutagenesis experiments. Sanger sequencing of exons 3 to 7 of the *ApoL1* gene in the patient's genome revealed a G2 ApoL1 genotype with homozygous rs143830837 alleles (N388del and Y389del) in the SRA-interacting domain of ApoL1 (Fig. 2). The *ApoL1* gene showed additional substitutions in the pore-forming domain, rs2239785 G/A (E150K) and rs136174 C/A (M228I), and in the membrane-addressing domain, rs136176 G/A (R255K) and rs73885316 C/A (N264K) (Fig. 2). The E150K, M228I, and R255K variants are actually present in the ApoL1 wild-type (WT) version used in our laboratory since the discovery of the trypanolytic potential of ApoL1 (4), and these variants do not affect ApoL1 activity. M228I and R255K are coded by the ApoL1 G3 genotype (16), and the trypanolytic activity of ApoL1 G3 was confirmed using plasma samples (17). Since previous experiments have indicated that mutations in the membrane-addressing domain can be deleterious to trypanolytic activity (8), we decided to check the activity of the N264K variant. Therefore, we experimentally introduced the N264K substitution in WT, G1, and G2 ApoL1s by site-directed mutagenesis and expressed these ApoL1 variants as recombinant proteins (Fig. S5). The N264K variants of WT, G1, and G2 ApoL1 variants knocked down the lytic activity against *T. b. gambiense* 1 TgsGP knockout (KO) parasites (Fig. 3). Our mutagenesis experiments also showed that the lytic activity of G1 ApoL1 against serum-resistant *T. b. rhodesiense* was completely destroyed by the N264K substitution. The same trend was observed with *T. b. brucei* ribSRA. This is a *T. b. brucei* strain in which SRA was genetically introduced and which thus ectopically expresses SRA (10).

DISCUSSION

We successfully sequenced the genome of a trypanosome in a dried blood spot from a HAT patient in Ghana. Using SNP and targeted gene analysis, we showed that the trypanosome is an atypical *T. b. gambiense* strain that lacks TgsGP for its defense against trypanolytic ApoL1 in human serum but has the *T. b. gambiense* 1-specific L210S substitution in its HpHb receptor. Given that the patient was anemic, reduced uptake of TLF1 by the trypanosome due to the mutated HpHb receptor may partially explain the resistance to human serum. However, ApoL1 can still enter the trypanosome via TLF2 and lyse trypanosomes that do not have the TgsGP serum resistance protein. We have shown that the N264K substitution in the membrane-addressing domain of the ApoL1 protein of the patient knocks down the trypanolytic activity against this atypical *T. b. gambiense* strain devoid of TgsGP. Interestingly, the intact *AUT1* gene in GHANA indicates that this strain has never harbored the *TgsGP* gene and that GHANA diverged from the common ancestor before the *T. b. gambiense* type 1 strains. Because GHANA had the mutation for the L210S substitution in its HpHb receptor, this also indicates that the *T. b. gambiense*-specific HpHb mutation existed before TgsGP was intro-

A**B**

WT	MEGAALLRVSVLCIWSALFLGVGVRAEEAGARVQQNVPSTDTGDPQSKPLGDWAAGTMDP
GHANA	MEGAALLRVSVLCIWSALFLGVGVRAEEAGARVQQNVPSTDTGDPQSKPLGDWAAGTMDP

ESSIFIEDAIKYFKEKVSTQNNLLLLLDNEAWNGFVAAAEPRNEADELRKALDNLARQMIM
ESSIFIEDAIKYFKEKVSTQNNLLLLLDNEAWNGFVAAAEPRNEADELRKALDNLARQMIM

KDKNWHDKGQQYRNWFLKEFPRLKSELEDNIRRLRALADGVQKVHKGTTIANVVSGLSIS
KDKNWHDKGQQYRNWFLKEFPRLKSELEDNIRRLRALADGVQKVHKGTTIANVVSGLSIS

GILTLVGMGLAPFTEGSSLVLEPGMELGITAALTGITSSTMDYGKKWWTQAQAHDLVIXSL
GILTLVGMGLAPFTEGSSLVLEPGMELGITAALTGITSSTIDYGKKWWTQAQAHDLVIXSL

DKLKEVREFLGENISNFLSLAGNTYQLTRGIGKDIRALRRARANLQSVPHASASRPVTEPI
DKLKEVREFLGENISNFLSLAGNTYQLTRGIGKDIRALRRARANLQSVPHASASRPVTEPI

SAESGEQVERVNEPSILEMSRGVKLTDPVAPVSFFLVLDVVYLVYESKHLHEGAKSETAEELK
SAESGEQVERVNEPSILEMSRGVKLTDPVAPVSFFLVLDVVYLVYESKHLHEGAKSETAEELK

KVAQELEEKLNI LN NNYKILQADQEL
KVAQELEEKLNI LN NNYKILQADQEL

388 | 389

FIG 2 (A) Nucleotide alignment of ApoL1 exon 7 of the patient (alleles 1 and 2) and the wild-type ApoL1 gene (ref; GenBank accession number [NM_003661.3](#)); (B) amino acid alignment of WT ApoL1 and ApoL1 of the patient (GHANA). Allele variants in panel A are indicated with the dbSNP codes, and Sanger

(Continued)

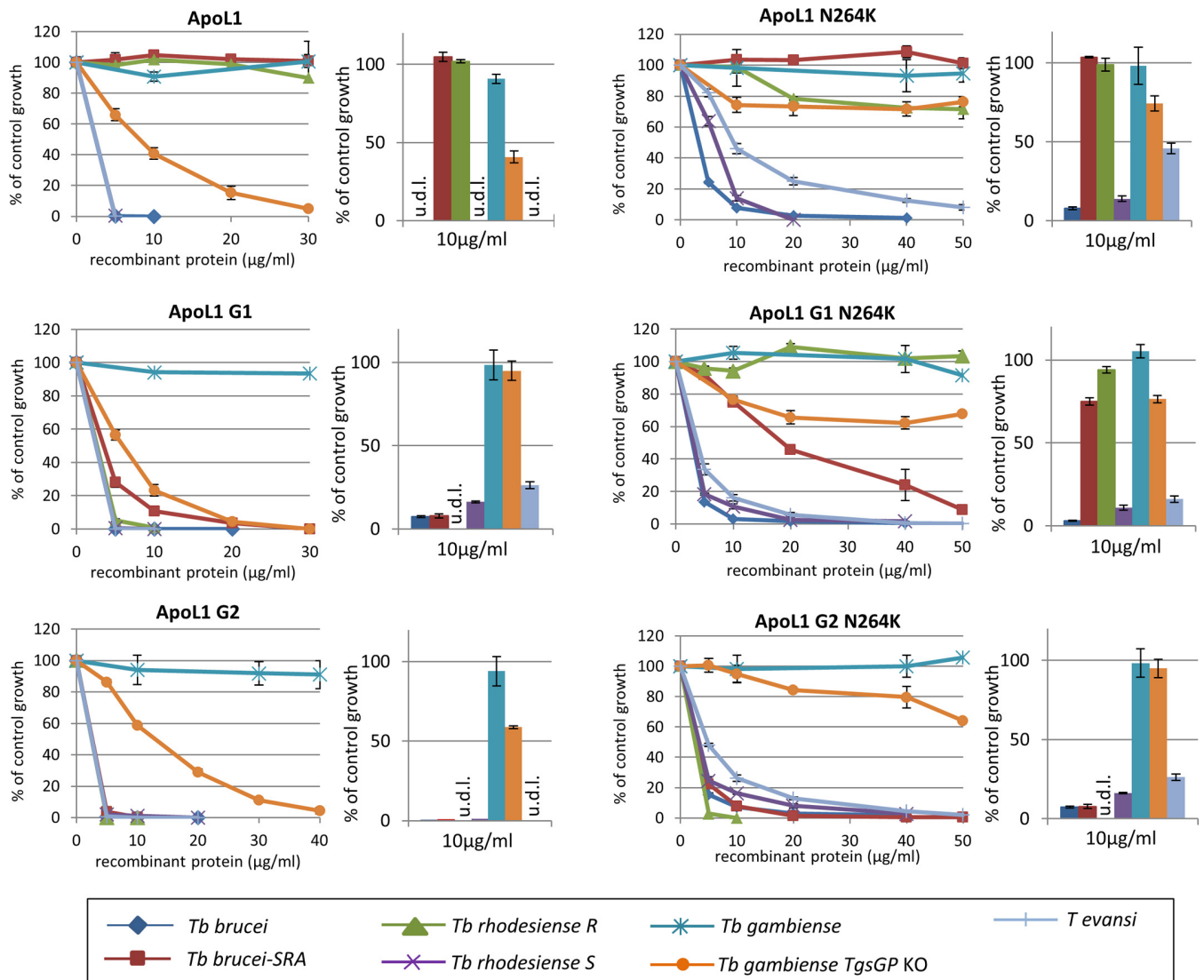


FIG 3 Trypanolytic activities of WT, G1, and G2 ApoL1s and the N264K variants. Shown is the growth of *T. b. brucei*, *T. b. brucei* ectopically expressing SRA (ribSRA), human-serum-resistant (R) and sensitive (S) clones of *T. b. rhodesiense*, *T. b. gambiense* 1, the *T. b. gambiense* 1 TgsGP knockout (KO), and *T. evansi* in complete growth medium supplemented with various concentrations of recombinant WT ApoL1 (A), ApoL1 G1 (B), and ApoL1 G2 (C), as well as the N264K variants ApoL1 N264K (D), ApoL1 G1 N264K (E), and ApoL1 G2 N264K (F). Histograms for the 7 strains in the presence of 10 µg/ml recombinant ApoL1 protein are shown for each ApoL1 variant. The physiological concentration of ApoL1 in human blood is approximately 10 µg/ml. Completely lysed trypanosomes in the histograms are indicated by “u.d.l.” (under the detection limit).

duced and before the monophyletic human pathogen *T. b. gambiense* type 1 emerged.

ApoL1 is targeted to membranes by its membrane-addressing domain (8). After ApoL1 is inserted into the lysosomal membrane, its pore-forming domain creates membrane pores that lead to trypanosome death (18). The N264K substitution in the membrane-addressing domain of G2 ApoL1 of the patient thus explains why he was infected with the atypical *T. b. gambiense*

strain devoid of TgsGP. In contrast, G2 ApoL1 N264K still efficiently lysed *T. b. brucei*, *T. evansi*, and *T. b. rhodesiense*. When we introduced the N264K substitution in G1 and G2 ApoL1 proteins, both variants could lyse *T. b. brucei* and *T. evansi*. The observation that N264K affects only the lytic activity of ApoL1 against *T. b. gambiense* devoid of TgsGP and not *T. b. brucei* and *T. evansi* is probably due to the lower inherent sensitivity of the *T. b. gambiense* TgsGP KO to ApoL1, caused by a decreased ApoL1 uptake

Figure Legend Continued

sequencing chromatograms are included for alleles rs73885316 C/A and rs14380837. The green, blue, and purple sequences in panel B represent, respectively, the pore-forming domain, the membrane-addressing domain, and the SRA-interacting domain of ApoL1 (8). Underlined sequences represent alpha helices. These helices are defined in reference 8; in the pore-forming domain, they were defined by homology modeling using the pore-forming domain of colicin A as the template. Amino acid substitutions or deletions, as well as the amino acid positions, are indicated in red.

in hypohaptoglobinemic serum and an accelerated degradation of the internalized ApoL1 (9). Interestingly, the N264K substitution also completely knocked down the lytic activity of G1 ApoL1 against *T. b. rhodesiense*, although N264K is located in the membrane-addressing domain of ApoL1 and not in the domain that binds to the *T. b. rhodesiense* resistance protein SRA. However, the N264K G2 ApoL1 could still lyse *T. b. rhodesiense*. G1 and G2 ApoL1 proteins have different mutations in the SRA-binding domain ablating the serum resistance of *T. b. rhodesiense*, the G1 variant being weaker than the G2 variant in lysing *T. b. rhodesiense*, presumably due to higher residual binding to SRA (13). Therefore, the higher susceptibility to N264K knockdown for the G1 variant in *T. b. rhodesiense* may result from the increased probability of interaction with SRA following a lowered ability to access the target membrane.

The G2 ApoL1 gene of the patient was characterized by a homozygote missense mutation (rs73885316 C/A) causing an N264K substitution in the membrane-addressing domain of ApoL1 and a homozygote 6-bp deletion (rs143830837) causing the N388del and Y389del deletions in the SRA-interacting domain of ApoL1. According to the 1000 Genomes Project, 31 individuals were heterozygous for the rs73885316 C/A allele, of which 8 individuals were heterozygous for the rs143830837 deletion; none were homozygous for rs73885316 C/A or rs143830837. This suggests that both mutations probably arose independently on different haplotypic backgrounds, given that they are only 368 bp apart. Therefore, the observation that the patient is homozygous for both rs73885316 (N264K) and rs143830837 (N388del and Y389del) is remarkable, as it could have happened only through convergent evolution (allowing the same mutations to arise in different haplotypes) or through recombination (allowing the mutations to be merged on the same haplotype). Ko et al. reported that the allele for the N264K ApoL1 variant is frequently observed (34%) in the Hadza population in Tanzania (16). Following the Hardy-Weinberg equilibrium, we expect 11.5% homozygous N264K ApoL1 variants in the Hadza population. Given that the Hadza live in an area where *T. b. rhodesiense* is endemic, our data thus suggest that about 11% of the people may be at increased risk of contracting HAT, since the N264K substitution knocks down the protective effect of G1 ApoL1 against *T. b. rhodesiense*. The G1 genotype is characterized by an S342G (rs73885319) and an I384M (rs71785313) substitution in the SRA-interacting domain of ApoL1, and both alleles are present in the Hadza population with a 5% frequency (16).

In conclusion, we report that the missense N264K substitution in the membrane-addressing domain of WT, G1, and G2 ApoL1s knocks down the lytic activity against *T. b. gambiense* strains devoid of the TgsGP serum resistance mechanism. Interestingly, the N264K substitution also knocks down the lytic activity of G1 ApoL1 against *T. b. rhodesiense*. Trypanosomes are ancient parasites that have existed together with human populations on the African continent through evolution. Our findings illustrate the sensitive interplay between ApoL1 genotypes and susceptibility to trypanosome infection (19).

MATERIALS AND METHODS

Ethics. Ethical clearance for the study was obtained from the Institutional Review Board (IRB) of the Institute of Tropical Medicine, Antwerp, Belgium (approval number IRB/AB/ac/108). No informed consent of the study participant could be given because the patient died before the results

of the molecular laboratory tests were available. The IRB gave approval to publish our findings without informed consent, as the patient is anonymized in the report.

Genome sequencing and SNP analysis. DNA from three 5-mm punches of dried blood of the patient was extracted from the filter paper using the QIAamp DNA microkit according to the manufacturer's instructions (Qiagen, Germany). One nanogram of genomic DNA was subjected to Illumina's Nextera XT library preparation, and the library was quantified with the KAPA library quantification kit (KAPA Biosystems, MA). The indexed library was sequenced at a final concentration of 33 pM in a MiSeq sequencer using a reagent v3 600-cycle cartridge (Illumina, CA). With Bowtie 2 under default settings (20), we first discarded the GHANA reads that mapped to human genome version 19 and then mapped the reads of GHANA and the publically available genome reads from the *T. b. gambiense* 1 strains DAL972 (21), Fontem strain 10, and 1829(Aljo) (22), the *T. b. gambiense* 2 strains TH126 and STIB386 (21), the *T. evansi* strains STIB810, E110, C13, KETRI2479, and STIB805 (23), and the West African *T. b. brucei* strains TSW187/78E, LM56clone6, TSW55, KP33clone16, PTAG129, B8/18cloneB, and SW3/87 (22) to the *T. b. brucei* TREU927 reference genome (24). The list of strains with country, host, and date of collection is presented in the supplemental material (see Table S1). Variants were called with SAMtools' mpileup function and bcftools (25). The resulting vcf file was filtered to retain only SNPs with read depths of ≥ 5 in all strains, an SNP quality of ≥ 50 , a mapping quality of ≥ 40 , and no significant strand bias ($P > 0.05$). For each strain, SNPs were concatenated to a FASTA sequence in which each SNP was represented as 2 bases, one for each allele, and invariant sites were subsequently removed. RAxML v8.1.17 was used to construct a maximum-likelihood tree with 1,000 bootstraps using a GTR CAT substitution model and the Felsenstein correction for ascertainment bias inherent to SNP alignments (26). To check for possible recombination events, a NeighborNet Split network of the SNP alignment was generated in Splitstree4 (27). The presence and sequence of the HpHb receptor, TgsGP, and SRA genes in the genomes was checked by mapping the sequencing reads against the FASTA sequences of these genes (NCBI accession number [JX143837](#) for the HpHb receptor gene, [FN555988](#) for TgsGP, and [Z37159](#) for SRA) using Bowtie2 and by visual inspection with the Integrated Genomics Viewer (IGV) (28).

TgsGP and HpHb receptor analysis. The presence of the TgsGP gene in GHANA was verified by PCR amplification with TgsGP internal primers reported by Radwanska et al. (29). The presence of a truncated AUT1 allele was verified as described by Felu et al. (12) but with the primers AUT1-Fwd (5' GTTGAGGATGAACAAACAAAGTTTGATG AAGG 3'), AUT1-Full-Rev (5' CATTTTAAATGTCGATACCTGTCGA CAAATCATATTCG 3'), and AUT1-Trunc-Rev (5' ATATTCGTATATA CACTCTCACAACACTCTCATATCATTATTATTATC 3'). The HpHb receptor gene was PCR amplified and Sanger sequenced as described elsewhere (9, 15). Successful DNA extraction from the dried blood on filter paper was verified by amplifying a part of the trypanosome's 18S rRNA gene and the patient's cytochrome *b* gene with primers described previously (30, 31).

ApoL1 genotyping and mutagenesis. To characterize the patient's ApoL1 protein, exons 3 to 7 of this gene were amplified using PCR as described previously (32). ApoL1 PCR products were cloned in pCR-Blunt vectors, and six to eight clones per exon were Sanger sequenced. Amino acid sequences of the exons were aligned against the ApoL1 reference sequence ([NM_003661.3](#), NCBI). ApoL1 G1, ApoL1 G2, and the N264K variants were obtained by site-directed mutagenesis of WT ApoL1 cloned in the pStaby expression vector (Delphi Genetics). Recombinant ApoL1 variants were expressed in *Escherichia coli* in fusion with the His tag and affinity purified on nickel columns (11). Trypanosome strains used in the lysis experiment were *T. b. gambiense* 1 ELIANE (clone LiTat1.3), *T. b. gambiense* 1 TgsGP KO, *T. b. brucei* Lister 427, *T. b. brucei* ribSRA, *T. b. rhodesiense* Etat1.2 R (serum-resistant phenotype) and S (serum-sensitive phenotype), and *T. evansi* RoTat 1.2. The transgenic cell lines *T.*

b. gambiense 1 TgsGP KO and *T. b. brucei* ribSRA were described earlier (9, 10). Bloodstream forms were diluted at 1×10^5 trypanosomes/ml (*T. b. brucei* and *T. b. rhodesiense*) or 2×10^5 trypanosomes/ml (*T. b. gambiense* 1 and *T. evansi*) in HMI (Hirumi Modified Escove [33]) medium containing 10% fetal calf serum (FCS) and 10% Serum Plus. Appropriate dilutions of the recombinant proteins were made in 20 mM acetic acid and added to the trypanosomes as 10% of the final volume. Lysis tests were performed in triplicate, and parasites were incubated overnight under standard culture conditions. Quantification was performed with the Cell-Titer Glo luminescence assay (Promega), and control growth conditions with addition of only acetic acid were used as the reference (100% growth).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.02198-15/-/DCSupplemental>.

Figure S1, TIF file, 0.1 MB.
Figure S2, PDF file, 0.3 MB.
Figure S3, PDF file, 0.3 MB.
Figure S4, PDF file, 0.4 MB.
Figure S5, TIF file, 3 MB.
Table S1, PDF file, 0.3 MB.
Table S2, PDF file, 0.3 MB.

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